

Amendments to the Specification

Please replace the paragraph on pages 10, starting at line 37, with the following amended paragraph:

Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482 (1981); by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970); by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. 85:2444 (1988); by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California; GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins and Sharp, Gene 73:237-244 (1988); Higgins and Sharp, CABIOS 5:151-153 (1989); Corpet, et al., Nucleic Acids Research 16:10881-90 (1988); Huang, et al., Computer Applications in the Biosciences 8:155-65 (1992), and Pearson, et al., Methods in Molecular Biology 24:307-331 (1994). The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, Current Protocols in Molecular Biology, Chapter 19, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995).

Please replace the paragraph on pages 11, starting at line 26, with the following amended paragraph:

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using the BLAST 2.0 suite of programs using default parameters. Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology-Information (<http://www.ncbi.nlm.nih.gov/>).

Please replace the paragraph on pages 11, starting at line 23, with the following amended paragraph:

This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some

positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

Please replace the paragraph on pages 12, starting at line 26, with the following amended paragraph:

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Natl. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance.

BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise regions of nonrandom sequences which may be homopolymeric tracts, short-period repeats, or regions enriched in one or more amino acids. Such low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, *Comput. Chem.*, 17:149-163 (1993)) and XNU (Claverie and States, *Comput. Chem.*, 17:191-201 (1993)) low-complexity filters can be employed alone or in combination.

Please replace the paragraph on pages 13, starting at line 9, with the following amended paragraph:

(c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Meyers and Miller, Computer Applic. Biol. Sci., 4:11-17 (1988) e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

Please replace the paragraph on pages 15, starting at line 24, with the following amended paragraph:

As used herein, often the designation of a particular polymorphism is made by the name of a particular restriction enzyme. This is not intended to imply that the only way that the site can be identified is by the use of that restriction enzyme. There are numerous databases and resources available to those of skill in the art to identify other restriction enzymes which can be used to identify a particular polymorphism, for example <http://darwin.bio.geneseo.edu> which can give restriction enzymes upon analysis of a sequence and the polymorphism to be identified. In fact as disclosed in the teachings herein there are numerous ways of identifying a particular polymorphism or allele with alternate methods which may not even include a restriction enzyme, but which assay for the same genetic or proteomic alternative form.

Please replace the paragraph on pages 16, under the heading "Description of the Figures" starting at line 8, with the following amended paragraph:

Figure 1 is a depiction of the porcine PRKAG3 nucleotide sequence (SEQ ID NO:1), including the amino ~~acides~~ acids, alternative polymorphic loci and their amino acid changes are identified.

Please replace the paragraph on pages 16, under the heading "Description of the Figures" starting at line 11, with the following amended paragraph:

Figures 2A (SEQ ID NO:11) and 2B (SEQ ID NO:12) depict the sequence of the 5' flanking region of the PRKAG3 gene including exon 1, exon 2 and novel intron sequence in between. Figure 2A is with SINE (11) and Figure 2B is without SINE (22). This sequence may be used to form additional primers. Bold designates direct repeats between the SINE; bold and italic designates exons of PRKAG3 gene (exon 1 and exon 2).

Please replace the paragraph on pages 18, starting at line 27, with the following amended paragraph:

The different marker genotypes of PRKAG3-199 are the result of a polymorphism within the PRKAG3 gene that results in a guanine to adenine transition at ~~nucleotide~~ nucleotide position 595, (SEQ ID NO:7) resulting in a change of the amino acid valine to isoleucine (amino acid number 199) (SEQ ID NO:8). This transition in turn generates a restriction site in allele 1 associated with lower glycogen, lactate and glycolytic potential. This site was also found to correlate with increased litter size when at least one copy was present.

Please replace the paragraph on pages 19, starting at line 24, with the following amended paragraph:

Thus, the invention relates to genetic markers and methods of identifying those markers in an animal of a particular breed, strain, population, or group, whereby the female animal is more likely to produce a litter that is significantly increased in size (number) above the mean for that particular breed, strain, population, or group. Similarly the method may be used to identify animals that are more likely to yield ~~meat~~ meat of preferred meat quality.

Please replace the paragraph on pages 30, starting at line 29, with the following amended paragraph:

Yet another technique includes an Invader Assay which includes isothermal amplification that relies on a catalytic release of fluorescence. See Third Wave Technology at www.twt.com.

Please replace the paragraph on pages 38, starting at line 29, with the following amended paragraph:

Pedigree, linkage and QTL mapping: We have generated an intercross between Berkshire and Yorkshire (BxY) pig breeds yielding 525 F₂ offspring and used this pedigree to map QTL for meat quality (Malek et al., 2001) using an interval mapping method (Haley et al., 1994). In this cross, the Berkshire breed was chosen as it is regarded as having very good meat quality, particularly in terms of pH, color, water holding capacity and tenderness. The *PRKAG3* gene was mapped to the BxY family linkage map using the CRI-MAP (version 2.4) mapping program (Green et al., 1990). The interval mapping method (Haley et al., 1994) including the *PRKAG3* site information was used to map the QTL for meat quality for pig chromosome 15 (SSC 15) (Figure 4-3). The QTL effects were estimated and represent the average Berkshire allelic effect compared to the average Yorkshire allelic effect.

Please replace the paragraph on pages 38, starting at line 29, with the following amended paragraph:

PCR, RT-PCR, RACE and Polymorphism Discovery: Based on *PRKAG3* pig gene sequence available in GenBank (AF214521), we designed primers to amplify the entire coding regions of the *PRKAG3* gene. The PCR reactions were performed using 12.5 ng of porcine genomic DNA, 1.5mM MgCl₂, 0.125 mM dNTP, 0.3 µM of each primer and 0.35 U *Taq* DNA polymerase (Promega, Madison, WI) and PCR buffer (10 mM Tris-HCl, 50 mM KCl, and 0.1% Triton®X-100) in a 10-µl final volume. The reverse transcription of total RNA (3.5µg) was performed by random hexanucleotide priming and Superscript II (GIBCO/BRL, Rockville, MD) according to the manufacturer's protocol (primers: Set A, forward 5'ATGAGCTTCCTAGAGCAAGGAG 3' (SEQ ID NO:13) and reverse 5'CAGGTCTCAATCTTATGTTCTTC 3' (SEQ ID NO:18); set B, forward 5'CGTCCGAGCGGCACCTTTGT 3' (SEQ ID NO:19),

and reverse 5' AAGGTTCCAAGGTTCTCAGGC 3' (SEQ ID NO:20)). The 5' Rapid Amplification of cDNA Ends (RACE) experiments were performed using FirstChoice RLM-RACE kit (Ambion, Austin, TX) according to the manufacturer's instructions followed by sequencing of the PCR products (gene specific primers: outer 5'CCCACGAAGCTCTGCTTCTT 3' (SEQ ID NO:17), and inner 5'TCCTTGCTCTAGGAAGCTCAT 3' (SEQ ID NO:21)). The amplicons were sequenced using dye terminators (PE Applied Biosystems, Foster City, CA) on an ABI 377 automated sequencer. We used Sequencher software (Gene Codes Corporation, version 4.0.5, Ann Arbor, MI) to assemble the sequences and to identify polymorphisms.

Please replace the paragraph on pages 38, starting at line 17, with the following amended paragraph:

Genotyping and PCR-RFLP analysis: The region flanking each analyzed missense mutation was amplified using the same pair of primers for the T30N and G52S substitutions (forward 5' ATGAGCTTCCTAGAGCAAGGAG 3' (SEQ ID NO:13) and reverse 5' GGCTGCATGATGTTATGTGCCT 3' (SEQ ID NO:14)) and a different pair for I199V (forward 5' GGAGCAAATGTGCAGACAAG 3' (SEQ ID NO:16) and reverse 5' CCCACGAAGCTCTGCTTCTT 3' (SEQ ID NO:17)). After digestion with *Bsa*HI (for I199V), *Hph*I (for G52S) and *Sty*I (for T30N) restriction enzymes, the digested PCR products were separated on 4% NuSieve agarose (FMC, Rockland, ME) gels and stained with ethidium bromide. For the SINE polymorphism, PCR amplification (primers: forward 5' GAAACTCTTCTCCCCACAGAC 3' (SEQ ID NO:15) and reverse 5' GGCTGCATGATGTTATGTGCCT 3' (SEQ ID NO:14)) was followed by separation of the products on a 1% agarose (AMRESCO, Solon, OH) gel. After genotyping for these polymorphisms, all the animals with haplotype 2 (Table 6) were also genotyped for the R200Q substitution in order to increase the chance of finding the RN⁻ or 200Q allele (see Milan et al., 2000). Two homozygotes for the 200Q allele and four carriers were found and these were removed from further statistical analyses so that the RN⁻ mutation did not affect our analysis of the other substitutions. For the R200Q substitution we used the same primers as for the I199V mutation and the digestion was performed with the *Bsr*BI restriction enzyme. As a final check, a random sample of about 100 animals with different haplotypes was also scored for the R200Q substitution, but none of animals carried the 200Q allele.

Please replace the paragraph on pages 41, starting at line 27, with the following amended paragraph:

Marker Development and Linkage Mapping: Several significant QTL were detected on SSC15 (Malek et al., 2001) in the region where the *PRKAG3* gene was located (Milan et al., 2000), between the markers SW1683 and SW1983 (Figure 1 3). These included QTL for average glycogen content and glycolytic potential which have been reported (Milan et al., 2000) to be affected by the *PRKAG3* 200Q allele as well as the traits 24 hr ham and loin pH and 24 hr loin Hunter L values (light reflectance). The favorable allele at this QTL, which interestingly, has an additive effect (the RN⁻ mutation is dominant) was derived predominantly from the Berkshire breed (generally regarded as having very good meat quality) as expected (Table 1). The *PRKAG3* gene was the unique candidate gene in this area, based on the recent development of the BAC contig in the porcine RN region (Milan et al., 2000), the high degree of linkage order conservation of the porcine map in this area with the human transcript map (Jeon et al., 2001)

and the recently developed human genome map (Lander et al., 2001). We first tested the founder animals, two Berkshire sires and nine Yorkshire dams, for the published RN⁺ substitution (R200Q). All the founder animals had the rn⁺ allele (200R). By sequencing the entire coding region of the *PRKAG3* gene in BxY family founders and in four F₃ individuals with extreme values for meat quality, we identified three missense mutations. These are the T30N and the I199V substitutions previously described (Milan et al., 2000) and a new missense mutation (G52S). Another non-synonymous substitution (P53L) found by Milan et al. (2000) was not found to be segregating in the founders of the BxY family where they were all 53P. Due to the lack of information on the 5'UTR, we used RACE in order to find the complete 5' flanking sequence and gene organization in that region. An intronic SINE polymorphism was discovered starting 79bp upstream of the start codon but this was present only in three Yorkshire grandams. Based on the differences in allele frequency of each site between the founders of the intercross family, we considered the G52S and I199V substitutions as the most likely candidates for the meat quality QTL reported previously. Using the I199V substitution we mapped the *PRKAG3* gene in the BxY linkage map to a position below the broad peak(s) of the QTL for glycogen, lactate and glycolytic potential and 24 hr pH (Figure-4_3). After adding the *PRKAG3* I199V information the map length and marker order on SSC 15 was the same as in Malek et al. (2001). Re-analysis of the QTL including *PRKAG3* I199V (Figure-4_3) caused small changes in the F value and the location of the QTL peaks on SSC 15 (from 0 to 3 cM) when compared with the results of Malek et al. (2001).

Please replace the paragraph on pages 45, starting at line 7, with the following amended paragraph:

The haplotype substitution effects for each line and across lines were calculated as the deviation from the average of the four haplotypes (Figure-2_4). Across and within line analyses showed bigger differences between haplotypes for ham pH and color measurements than for traits of the loin. For ham pH, across and within line analyses showed haplotype 3 having the highest effect which was significantly different from each of the other haplotypes in the across lines analysis ($p < .0005$) and from at least one other haplotype in each individual line analysis ($p < .05$). Haplotype 2 was the next best for most of the traits and lines with haplotypes 1 and 4 tending to be the worst with respect to meat quality. This hierarchy is not evident in the Berkshire population, where significant differences are only seen with haplotype 4 which has the lowest value, corresponding to the across lines result. The non-significant results in Berkshire are likely to be due in part to the low level of polymorphism in this breed and the concomitant very low number of observations for haplotypes 1 and 4. The estimate for haplotype 4 in the Duroc Synthetic population appears to be different to that in the other lines (especially for ham pH

where it is significantly higher than haplotype 2 ($p < .05$) and haplotype 1 ($p < .01$), but the frequency of haplotype 4 in this population was very low (0.07). The synthetic nature of this line (though its inception was six generations ago) also provides the opportunity for extended linkage disequilibrium to be present, increasing the chance for linked loci to contribute to the haplotype substitution effects.

Please replace the paragraph on pages 48, starting at line 5, with the following amended paragraph:

The difference between haplotype 4 and haplotype 2 is only at the G52S site. The effects of haplotype 4 and 2 are significantly different for pH and Minolta L scores in both ham and loin in the across lines analysis and for several traits of the individual lines, most notably the Large White. Haplotype 2 (which contains 52S and encodes a serine) is favorable over haplotype 4 (which contains 52G and encodes a glycine). This is the opposite of what was found in the BxY study where 52G was predicted to be the favorable allele. Strong linkage disequilibrium with the I199V site, due to the limited number of founders of the F₂, may have masked the true effect of the G52S substitution in this population. Interestingly, the individual analysis of G52S did not show any effect for most traits and lines. That analysis compares haplotype 2 with the other three combined. It can be seen from Figure-2_4 that a mixture of the other three haplotypes can, depending on haplotype frequencies, result in a mean value close to that of haplotype 2 so that a difference would not be detected when G52S is analyzed individually, which points out the value of haplotype based analysis.

Please replace the paragraph on pages 64, under the heading "Primers", lines 8-9, with the following amended paragraph:

RF1 - 5'ATG AGC TTC CTA GAG CAA GGA G 3' (SEQ ID NO:13)
RN52R2 - 5'GGC TGC ATG ATG TTA TGT GCC T 3' (SEQ ID NO:14)

Please replace the paragraph on pages 65, under the heading "Primers", lines 21-22, with the following amended paragraph:

RP1F -5' GAA ACT CTT CTC CCC ACA GAC 3' (SEQ ID NO:15)
RN52R2 -5' GGC TGC ATG ATG TTA TGT GCC T 3' (SEQ ID NO:14)

Please replace the paragraph on pages 66, under the heading "Primers", lines 18-19, with the following amended paragraph:

RF1 -5'ATG AGC TTC CTA GAG CAA GGA G 3' (SEQ ID NO:13)
RN52R2 - 5'GGC TGC ATG ATG TTA TGT GCC T 3' (SEQ ID NO:14)

Please replace the paragraph on pages 67, under the heading “Primers”, lines 27-28, with the following amended paragraph:

RNF - 5' GGA GCA AAT GTG CAG ACA AG 3' (SEQ ID NO:16)

RNR - 5' CCC ACG AAG CTC TGC TTC TT 3' (SEQ ID NO:17)